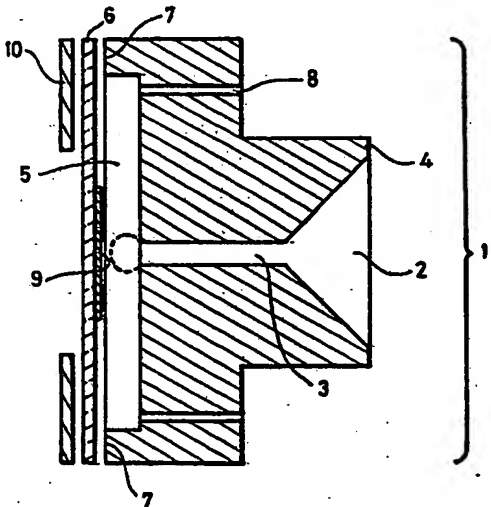


PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : B01L 3/00, G01N 33/52		A1	(11) International Publication Number: WO 92/07655 (43) International Publication Date: 14 May 1992 (14.05.92)
(21) International Application Number: PCT/GB91/01896 (22) International Filing Date: 30 October 1991 (30.10.91) (30) Priority data: 9023965.8 30 October 1990 (30.10.90) GB 9024305.6 8 November 1990 (08.11.90) GB (71) Applicant (for all designated States except US): HYPO-GUARD (UK) LIMITED [GB/GB]; Dock Lane, Melton, Woodbridge, Suffolk IP12 1PE (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : COX, Stephen, John [GB/GB]; 26 Cliff Road, Felixstowe, Suffolk IP11 9PJ (GB). LYDFORD, Thomas [GB/GB]; 29 Chester Road, Felixstowe, Suffolk IP11 9AH (GB).			(74) Agent: DUMMETT, Thomas, Ian, Peter; Dummett Copp & Co., 14 The Square, Martlesham Heath, Ipswich, Suffolk IP5 7SL (GB). (81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU*, TD (OAPI patent), TG (OAPI patent), US. Published <i>With international search report.</i>
(54) Title: COLLECTION AND DISPLAY DEVICE			
			
(57) Abstract <p>The present invention relates to a device for assessing a fluid sample, characterised in that it comprises: a) a fluid receptor means adapted to receive a sample of a fluid from an external source; b) a substantially closed chamber adapted to receive fluid from the receptor means by means of fluid flow connection therebetween, the chamber having at least part of a wall thereof provided by a member carrying one or more reagents adapted to respond to one or more components of the fluid sample and adapted to give an indication of that response which can be detected from the exterior of the chamber. The invention also provides a method for assessing a fluid sample in which the sample is placed in the receptor of a device of the invention and allowed to contact the reagent(s) within the chamber to provide a colour or other reaction which can be detected externally through the wall of the chamber.</p>			

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LJ	Liechtenstein	SU+	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark				

TITLE: COLLECTION AND DISPLAY DEVICE

The present invention relates to a collection and display device, notably to one for receiving a sample of a fluid and
5 for presenting that to a reagent pad integral with the sample receiving device.

BACKGROUND TO THE INVENTION:

10 Samples of blood and other bodily fluids, for example urine, sputum etc., are frequently collected and analyzed to monitor the state of health of a human or other mammal or to identify the presence of an organism. Typically, the sample is collected in one vessel and then transferred to a
15 separate reagent unit where a colour or other visible or non-visible indicator is developed by interaction of the sample with one or more reagents. The reagent unit or part thereof is then discarded, often with at least some of the sample still carried thereon in a state where it can contact
20 the user and/or other parts of the test equipment. Such systems are cumbersome and carry the risk that there will be cross-infection or contamination between samples and the risk of infection of the user from the samples or the discards.

25

It has therefore been proposed to provide the necessary reagents in a pad upon a disposable carrier strip so that the test is carried out by applying the fluid to the reagent pad, monitoring the pad for the required colour or other
30 change and then disposing of the pad and any remaining sample. This reduces the risk of cross-contamination between samples prior to monitoring the colour or other change in the reagent pad. However, there remains the problem of cross-contamination at the instrument where the
35 response of the reagent is assessed, since the reagent pad

and the fluid carried on it are exposed and can be contacted by the user or by exposed parts of the test apparatus.

This can be reduced by providing each patient with their own reagent response assessment unit, but this is impractical where a large number of people are being monitored at a single site, for example in a hospital. Furthermore, many people, notably the blind, infirm or very young, have difficulty in operating such a system, thus requiring that the tests on the samples of their bodily fluids be carried out by a third party. This is inconvenient and re-introduces the risk of cross-contamination, especially where such tests are carried out at a central location to reduce the costs of having to provide individual test units.

We have devised a sample collection and reagent holder system which reduces the above problems. Since the system of the invention does not expose the collected sample, as is the case with current sample reagent test strips and the like, it is possible to monitor the changes in the reagent at a central location with reduced risk of cross-infection. Due to its combined function as a sample collector and reagent holder, the system of the invention readily lends itself to use by the blind, infirm or young.

SUMMARY OF THE INVENTION:

Accordingly, the present invention provides an integral fluid sample collector and sample assessment device, which device is characterised in that it comprises:

- a. a fluid receptor means adapted to receive a sample of a fluid from an external source;
- b. a substantially closed chamber adapted to receive fluid from the receptor means by means of fluid flow connection therebetween, the chamber having at least part of a wall

- 3 -

thereof provided by a member carrying one or more reagents adapted to respond to one or more components of the fluid sample and adapted to give an indication of that response which can be detected from the exterior of the chamber.

5

- Preferably, the chamber has means to vent or accommodate air displaced by the fluid entering the chamber.

Preferably, the chamber and the fluid receptor are connected
10 by a capillary bore so that the sample is drawn by capillary action into the chamber.

Preferably, the device is in the form of a machined or moulded metal, glass or plastic unitary construction body
15 member comprising a cup or recess having an exposed open top into which the sample to be tested is placed. The cup or recess is connected by a bore to a chamber within the body which has one face thereof formed at least in part from a demountable generally planar member which carries the
20 reagent for the test to be carried out on or accessible from one face thereof and adapted to provide a visual display of the response from the reagent to the sample at the other face thereof. The invention is not however limited to visual display of the response. It may be possible for the
25 response to be detected as a response outside the visible spectrum, for example in the infra-red or ultra-violet spectrum. For convenience, the invention will be described hereinafter in terms of a reagent system which develops a colour in response to contact with the bodily fluid.

30

The device of the invention is of especial application in testing blood samples for glucose and for convenience, it will be described with respect to this preferred use. However, it will be appreciated that the device can be used
35 to test for one or more components in a wide range of other

bodily fluids, for example blood or glucose in urine.

Preferably, the device is in the form of a generally cylindrical body having the cup or recess located at one end thereof with an axial bore leading to an axial chamber having the demountable member forming either an axial or transverse wall thereof. It is, however, preferred that the device have a diameter larger than its axial length and that the chamber have its transverse end wall remote from the axial inlet bore provided with the demountable member.

The cup or recess which is to act as the sample receptor means can be of any suitable size and shape. However, it will usually be preferred that the exposed open top to the cup or recess have an upstanding rim so that a user can present a finger carrying a drop of blood thereon to the open end and can draw the tip of the finger over the upstanding rim to aid transfer of the drop of blood from the finger tip into the cup or recess. Typically, the cup or recess will have a generally circular cross-section and will be formed by drilling or moulding an axial bore into one end of the body of the device.

The body member is provided with a bore which is to transfer the sample from the cup or recess to the chamber within the body. The bore is preferably a straight axial bore which connects the base of the cup or recess with the inlet to the chamber. Preferably, the bore is provided as a bore moulded or drilled into the body member with its axis substantially co-incident with the longitudinal axis of the body member. However, the bore may be provided by a length of a metal, for example stainless steel, capillary bore tube moulded integrally with the body member.

As indicated above, the bore is preferably a capillary bore

- 5 -

so that the blood sample is drawn into the chamber from the cup or recess. However, the bore need not be a capillary bore and the blood sample can be caused to flow under the influence of gravity between the cup and the chamber. Thus, 5 the bore can have a diameter of from 0.25 to 2.5 mms, notably from 0.5 to 1.5 mms. For convenience, the invention will be described hereinafter in terms of a capillary bore.

10 The chamber can be of any suitable shape or size and is conveniently formed during the moulding or machining of the body member so that it is a generally cylindrical chamber with its axis substantially co-incident with that of the body member. As indicated above, it is preferred that the 15 chamber have an open end face, for example by being formed by drilling a suitable recess axially into the end face of the body member opposite to that where the sample receptor cup is located. However, the chamber can be formed with the open face as part of the side wall of the chamber.

20 For convenience, the invention will be described hereinafter in terms of a generally cylindrical body member having the receptor cup at one end thereof and with the open face to the chamber at the other end, the cup, capillary bore and 25 chamber all being located with their longitudinal axes substantially co-incident, whereby the device is radially symmetrical.

The chamber receives the sample through an inlet from the 30 capillary bore, which is preferably merely the outlet to the bore. The volume of the chamber is selected so that sufficient fluid is drawn into the chamber to activate the reagent(s) in the demountable member to the desired extent. The chamber can have an axial dimension which is 35 sufficiently small so that the fluid entering the chamber

flows by capillary action over the internal faces of the chamber and onto the inner face of the demountable member forming the fluid testing member so as to ensure uniform wetting of the member with the fluid to be tested. However, where it is necessary to employ dimensions which do not achieve this, for example due to manufacturing restrictions, it may be necessary to achieve the spreading of the fluid by "flicking" the device transversely or axially to aid transfer of the sample through the capillary bore and onto the surface of the test member.

Typically, the chamber will have a transverse diameter to axial depth ratio of from 12:1 to 5:1. It is also preferred that the axial depth of the chamber be from 0.5 to 1.5 mm to ensure adequate spread of blood or other fluid over the walls of the chamber.

Alternatively, the chamber can be dimensioned so that the fluid will form a droplet at the chamber end of the capillary bore. The droplet can then be detached to fall at an accurately known position on the test member surface forming part of the opposite wall. If required, the outlet to the capillary bore can be provided with a sharp rim to aid separation of the droplet from the bore outlet and/or the walls of the chamber adjacent the bore outlet can be given a surface coating of a material which is not readily wet by the fluid entering the chamber. For example, the internal surfaces of the chamber can be given a coating of a polytetrafluoroethylene polymer or part of the chamber walls can be formed from such a material.

By forming the chamber so that the fluid forms a droplet at the outlet to the capillary bore rather than spreading over the inner walls of the chamber, the droplet falls upon a restricted area of the chamber wall opposed to the outlet of

- 7 -

the capillary bore rather than uniformly wetting the walls of the chamber. It is thus possible to limit the lateral spread of the droplet over the test member and to concentrate it at a given location. We have found that this
5 enables satisfactory results to be achieved with a smaller sample than hitherto, for example to use from 30 to 70% of the sample hitherto considered necessary.

Typically, the droplets formed at the outlet to a tube have
10 a diameter of from 1 to 5 times the internal bore of the tube outlet. Therefore, where transfer of the fluid from the capillary bore to the test member is achieved by detachment of a droplet, it is preferred that the chamber have an axial depth of from 1 to 5 mm. If required, the
15 droplet formed at the end of the bore can be detached by rapping the device sharply, for example by flicking it with a finger or tapping it sharply onto a surface.

It will be appreciated that the axial depth of the chamber
20 may not be sufficient, for example due to manufacturing requirements, to permit the droplet to form completely and detach from the capillary bore outlet. In such a case, the meniscus of the fluid may contact the opposed face of the test member so that the fluid then forms a bridge between
25 the member surface and the outlet from the capillary bore. Again, it may be necessary to flick the device axially or transversely to achieve contact between the meniscus and the surface of the test member.

30 The chamber is preferably also provided with means whereby air displaced by the fluid as it enters the chamber can be accommodated or vented, notably where the fluid reaches the reagent pad by wetting the walls of the chamber. The walls of the chamber can be formed with a bellows or flexible
35 section to allow the internal volume of the chamber to be

increased to achieve this. However it is preferred to vent the displaced air from the chamber so as to retain a simple and substantially rigid structure for the device. Preferably, that radial wall of the chamber adjacent the fluid inlet to the chamber is provided with air vents, for example simple radial or axial bores in the chamber wall. The optimum number and size of such bores can readily be determined by simple trial and error tests. It is preferred that such air vent bores have a diameter which is sufficiently small to prevent capillary action drawing fluid into those bores.

As stated above, at least part of one wall of the chamber is provided by a test member incorporating one or more reagents to respond to one or more components in the sample being assessed. The reagents can be any of those conventionally used to test blood or other fluids and can be incorporated into the test member as a surface pad on one face of the member or can be impregnated into the material from which the member is made so that the fluid can access the reagents when it contacts the surface of the test member. The test member is one which preferably develops some visual response to the component of the fluid being assessed and this response is viewed from the outside of the chamber, for example by forming the test member as a transparent or translucent sheet carrying the reagent pad on one face thereof.

The test member can be formed as a sheet member, optionally in a suitable support frame, which is clamped or otherwise affixed to the open face of the chamber. However, it is particularly preferred to form the test member as a disc of sheet material secured to the chamber by adhesive, and to provide the reagent as a pad located generally centrally upon the internal face of that disc. The disc is applied to

the end wall of the chamber which is formed with a circular aperture through which the fluid can spread to wet the inner face of the disc. The disc preferably has a blanking or opaque annular member or component so that the area of the disc visible from the outside of the chamber coincides with the area of the reagent pad on the inside of the disc. In this way the position of the area of the device of the invention to be inspected to monitor the colour or other change in the reagent pad can be accurately predicted. This aids mechanical observation of the change using optical or other means at a central processing location, rather than relying on visual inspection. Furthermore, such a construction is of especial benefit when the fluid is applied to the reagent pad as a droplet detaching from the capillary bore outlet as described above.

The device of the invention readily lends itself to manufacture as a plastic moulding to which a standard shape and form of reagent disc can be applied over the open end face of the chamber to provide a closed chamber into which the blood or other sample is drawn automatically by capillary action from the sample receptor cup. The sample is thus retained within a closed environment and the risk of cross-contamination between samples is much reduced. The sample in the device can then be assessed mechanically with reduced risk of cross-contamination at the test device, yet is simple and easy for the aged or infirm to use. Since the device can be accurately located in a suitable test device and the position of the test member fixed with respect to the test device, the device of the invention can readily be used by the blind.

DESCRIPTION OF THE DRAWINGS:

A preferred form of the device of the invention will now be

- 10 -

described by way of illustration with respect to the accompanying drawings in which Figure 1 is an axial cross-section through the device; and Figure 2 is an exploded perspective view of the components of the device.

5

DESCRIPTION OF THE PREFERRED EMBODIMENT OF THE INVENTION:

The device comprises a cylindrical housing member 1 injection moulded from a suitable plastic, for example a polystyrene; or machined from a metal such as stainless steel; or glass. At one end, the housing is formed with a sample receiving cup 2 connected to an axial capillary bore 3. The cup 2 has a rim 4 against which a user can draw his fingertip so as to transfer a drop of blood into the cup 2. Capillary bore 3 connects the base of cup 2 with the chamber 5 formed in the other end of the device. Chamber 5 has an open end face which is closed by applying an adhesive disc 6 to the annular rim 7 of the chamber. The chamber 5 is vented to the atmosphere by axial vent bores 8. Preferably, the housing, chamber, bore, cup and vent bores are formed symmetrically about the longitudinal axis of the housing.

The disc 6 carries substantially centrally thereon a reagent pad 9 and disc 6 is formed from a suitable transparent plastic so that the outer face of pad 9 can be seen through the material of the disc. A masking annular disc 10 is affixed to the outer face of disc 6 which serves both to mask the outer edge of the disc 6 and to support the central area of the disc. In an alternative form of disc 6, the reagent can be impregnated into the material of the disc and the masking rim 10 can be an integral part of the construction of the disc as opposed to being a separate component as shown.

35 In use, a user wipes his finger across the rim of cup 2 to

- 11 -

transfer a drop of blood into the cup. The blood travels along capillary bore 3 due to capillary action and either spreads over the internal walls of chamber 5 to wet the reagent pad 9 or forms a droplet (shown dotted in Figure 1) which detaches to fall directly onto the reagent pad 9. The blood sample is contained within chamber 5 and there is little risk of escape of the blood to contaminate the user, other samples or any test machine in which the response of the reagent is assessed. The reagent responds to one or more of the components in the blood in the usual manner and this response can then be observed through the circular viewing aperture in rim 10 from outside the container. Again, this response can be viewed without the need to remove the blood from chamber 5, further reducing the risk of cross-contamination.

Once the response has been generated, it can be observed and the device then discarded. Since the device is of known shape and dimensions and the location of the viewing aperture in rim 10 are accurately known, the device can readily be mounted in a suitable receptacle in a response monitoring device so that the outer face of disc 6 can be observed at the position of pad 9. The device can thus readily be handled mechanically where large numbers of samples are to be processed, or the device can be readily handled by a blind or infirm person to locate it in a monitoring device.

The invention thus also provides a method for testing a fluid sample for the presence of a component or property therein, which method is characterised in that the sample of fluid is applied to the receptor of a device as claimed in any one of the preceding claims, the fluid is allowed to flow through the bore to the chamber and to contact the reagent(s) carried by the wall thereof; and observing the

- 12 -

response of the reagent(s) to the fluid externally through the wall of the chamber.

5

10

15

20

25

30

35

CLAIMS:

1. A device for assessing a fluid sample, characterised in that it comprises:
 - 5 a. a fluid receptor means adapted to receive a sample of a fluid from an external source;
 - b. a substantially closed chamber adapted to receive fluid from the receptor means by means of fluid flow connection therebetween, the chamber having at least part of a wall
 - 10 thereof provided by a member carrying one or more reagents adapted to respond to one or more components of the fluid sample and adapted to give an indication of that response which can be detected from the exterior of the chamber.
- 15 2. A device as claimed in claim 1, characterised in that the device is in the form of a machined or moulded metal, glass or plastic unitary construction body member comprising a cup or recess having an exposed open top into which the sample to be tested is placed, the cup or recess being
- 20 connected by a bore to a chamber within the body which chamber has one face thereof formed at least in part from a demountable generally planar member which carries the reagent for the test to be carried out on the sample, which reagent is located on or accessible from the chamber
- 25 adjacent face of the member and is adapted to provide a visual display of the response from the reagent to the sample at the other face thereof.
3. A device as claimed in either of claims 1 or 2,
- 30 characterised in that the chamber is provided with means to vent or accommodate air displaced by the fluid entering the chamber.
4. A device as claimed in any one of the preceding claims,
- 35 characterised in that the chamber and the fluid receptor are

connected by a capillary bore.

5. A device as claimed in any one of the preceding claims, characterised in that it is of a generally cylindrical form
- 5 having the fluid receptor located at one end thereof and the chamber at the other end thereof and having an axial bore fluid flow connection between the chamber and the receptor.
6. A device as claimed in any one of the preceding claims,
- 10 characterised in that the reagent(s) are provided upon a demountable member forming at least part of a wall to the chamber.
7. A device as claimed in claim 6, characterised in that
- 15 the demountable member forms at least part of the transverse end wall of the chamber.
8. A device as claimed in either of claims 6 or 7, characterised in that the demountable wall comprises a
- 20 transparent or translucent planar member having applied thereto and accessible from one face thereof the reagents to respond to the fluid sample and adapted to provide a visual response to the fluid sample through the other face of the member.
- 25
9. A device as claimed in any one of the preceding claims, characterised in that the chamber has an internal transverse diameter to axial depth ratio which is from 12:1 to 5:1.
- 30 10. A device as claimed in claim 1, characterised in that the bore has a diameter of from 0.25 to 2.5 mms and the chamber has an axial depth of from 0.5 to 5 mms.
11. A device as claimed in any one of the preceding claims,
- 35 characterised in that the reagent(s) respond to glucose in

- 15 -

a blood sample.

12. A device as claimed in any one of the preceding claims, characterised in that the means for responding to the fluid
5 sample comprises a transparent or translucent substrate adapted to be removably attached across an open end face of the chamber, the substrate carrying one or more test reagents applied to the chamber-directed face thereof and an
10 annular disc of an opaque material surrounding the reagent(s) so as to restrict the development of the response to the fluid sample to a predetermined area of the substrate.

13. A device as claimed in claim 1 substantially as
15 hereinbefore described with respect to and as shown in the accompanying drawings.

14. A method for testing a fluid sample for the presence of a component or property therein, which method is
20 characterised in that the sample of fluid is applied to the receptor of a device as claimed in any one of the preceding claims, the fluid is allowed to flow through the bore to the chamber and to contact the reagent(s) carried by the wall thereof; and observing the response of the reagent(s) to the
25 fluid externally through the wall of the chamber.

15. A method as claimed in claim 14, characterised in that the fluid flows through the bore due to capillary action and forms a drop or partial drop at the chamber end of the bore
30 which contacts the reagent carrying surface of the wall of the chamber so as to wet that wall.

16. A method as claimed in either of claims 14 or 15, characterised in that the fluid is blood and the reagent(s)
35 give a colour response to the glucose content of the sample.

1/1

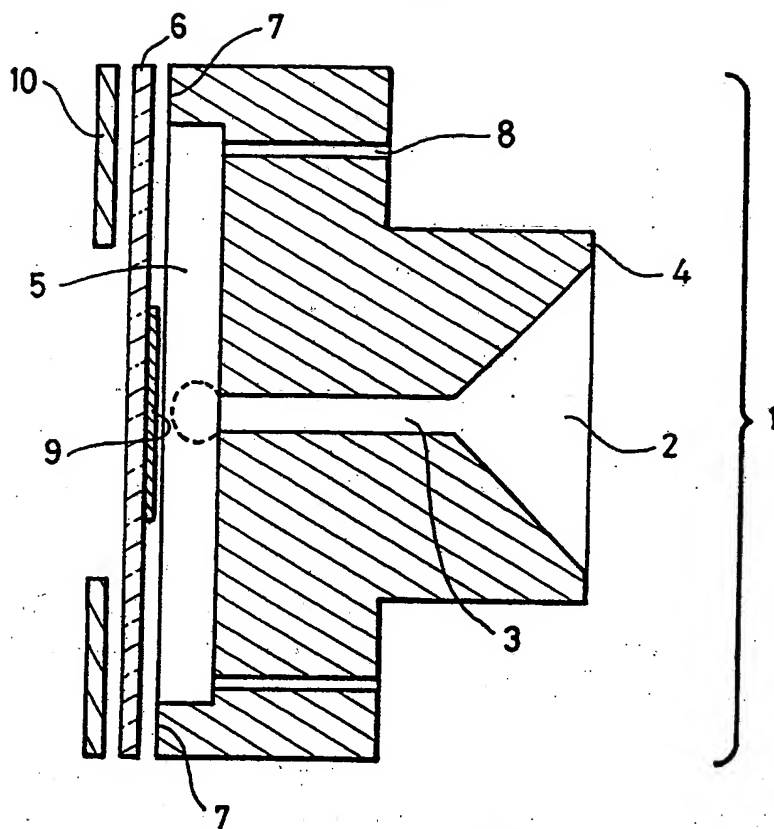


Fig. 1

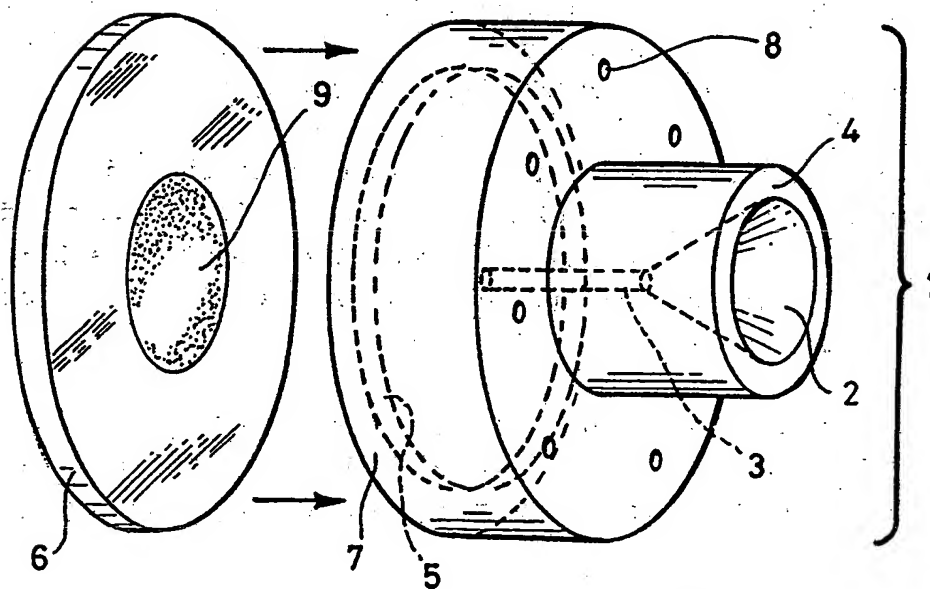


Fig. 2

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01896

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC5: B 01 L 3/00, G 01 N 33/52		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC5	B 01 L; G 01 N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP, A1, 0010456 (EASTMAN KODAK COMPANY) 30 April 1980, see page 7, line 15 - page 9, line 28; figure 5	1,3, 14
A		2,4-12, 15-16
X	EP, A1, 0388782 (QUANTAI BIOTRONICS INC) 26 September 1990, see page 3, line 11 - page 4, line 5; page 6, line 8 - line 31; figure 1	1,11,14, 16
A		2-10,12, 15
A	US, A1, 3915647 (RICHARD F. WRIGHT) 28 October 1975, see column 1, line 27 - line 40; claim 1	1-16
<p>[*] Special categories of cited documents:¹⁰</p> <p>^A document defining the general state of the art which is not considered to be of particular relevance</p> <p>^E earlier document but published on or after the international filing date</p> <p>^L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>^O document referring to an oral disclosure, use, exhibition or other means</p> <p>^P document published prior to the international filing date but later than the priority date claimed</p> <p>^T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>^X document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>^Y document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>^Z document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
24th January 1992	12 FEB 1992	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	MISS J. TAZELAAR	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	US, A, 4323536 (RICHARD L. COLUMBUS) 6 April 1982, see column 2, line 15 - line 45; column 5, line 65 - column 6, line 35; figure 5	1-4, 6-8, 14, 15
A		5, 9-12, 16

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 91/01896**

SA 52711

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 31/10/91. The European Patent office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0010456	30/04/80	AT-T- 1366	15/08/82
		CA-A- 1129498	10/08/82
		US-A- 4254083	03/03/81
		AT-T- 4249	15/08/83
		CA-A- 1119831	16/03/82
		CA-A- 1133059	05/10/82
		EP-A-B- 0010457	30/04/80
		EP-A-B- 0014797	03/09/80
		JP-C- 1196515	21/03/84
		JP-C- 1248146	16/01/85
		JP-A- 55059326	02/05/80
		JP-A- 55071942	30/05/80
		JP-A- 55074462	05/06/80
		JP-B- 58026968	06/06/83
		JP-B- 59021501	21/05/84
		US-A- 4233029	11/11/80
EP-A1- 0388782	26/09/90	NONE	
US-A1- 3915647	28/10/75	NONE	
US-A- 4323536	06/04/82	CA-A- 1160863	24/01/84
		EP-A-B- 0034049	19/08/81
		JP-A- 56125663	02/10/81

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12Q 1/54, 1/26, 1/28	A1	(11) International Publication Number: WO 92/22669 (43) International Publication Date: 23 December 1992 (23.12.92)
(21) International Application Number: PCT/GB92/01116 (22) International Filing Date: 19 June 1992 (19.06.92) (30) Priority data: 9113212.6 19 June 1991 (19.06.91) GB (71) Applicant (for all designated States except US): HYPO-GUARD (UK) LIMITED [GB/GB]; Dock Lane, Melton, Woodbridge, Suffolk IP12 1PE (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): GULLICK, Stephen, Peter [GB/GB]; 18 Deben Avenue, Martlesham, Ipswich, Suffolk IP5 7PQ (GB). (74) Agent: DUMMETT, Thomas, Ian, Peter; Dummett Copp & Co., 14 The Square, Martlesham Heath, Ipswich, Suffolk IP5 7SL (GB).		(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC (European patent), MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, RU, SD, SE, SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent), US. Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: REAGENT MIXTURES FOR GLUCOSE ASSAY (57) Abstract The present invention relates to a test reagent mixture composition comprising the enzymes glucose oxidase and peroxidase and a chromogen which interacts with the hydrogen peroxide from the oxidation of the blood glucose by the glucose oxidase, characterised in that the glucose oxidase and the peroxidase are present in proportions which provide from 300 to 700 International Units (IUs) of glucose oxidase and at least 20 International Units of peroxidase and in that the chromogen is present in an amount which provides from 12 to 20 grams of active chromogen per 500,000 International Units of glucose oxidase. Preferably, the composition is put up in a low molecular weight gelatin matrix and is impregnated into a micro-porous carrier membrane.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LJ	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TC	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

REAGENT MIXTURES FOR GLUCOSE ASSAY

The present invention relates to a reagent mixture, notably to a mixture of analytical reagents in a carrier gel which provides enhanced consistency of the colour generated with the elapse of time.

BACKGROUND TO THE INVENTION:

- 10 Blood samples are often assessed for the amount of glucose or some other constituent therein by reacting the blood with one or more reagents carried on a test stick or pad so as to develop a colour which can be observed by an operator. For example, a reagent pad can contain the enzymes glucose oxidase and peroxidase and o-tolidine as the chromogen which turns blue as the glucose oxidase oxidises glucose in the blood sample to gluconic acid and hydrogen peroxide. The hydrogen peroxide reacts in the presence of the peroxidase with the o-tolidine to give a blue colour whose intensity depends upon the amount of hydrogen peroxide released and hence the amount of glucose in the blood. The reagents are usually put up in a gel matrix, for example of a natural gel, for example a gelatin, or of a synthetic polymer, for example a polyvinylpyrrolidone.
- 25 However, problems arise in that the colour is affected by the time over which the blood sample is held in contact with the reagent pad, as well as the amount of blood in contact with the reagents. It is therefore customary for such tests to be carried out within a strictly monitored time schedule and the results are often of dubious value due to inaccuracies in observing the time schedule.

Surprisingly, we have found that the proportion of the reagents to one another in the matrix affects the period over which a consistent colour is produced by the interaction of the blood with the reagent. If the proportions in the mixture lie within

certain limits, the colour produced is sufficiently constant over a period of time for the need for strict adherence to a time schedule to be reduced.

5 SUMMARY OF THE INVENTION:

Accordingly, the present invention provides a blood test reagent mixture composition comprising the enzymes glucose oxidase and peroxidase and a chromogen which interacts with the
10 hydrogen peroxide from the oxidation of the blood glucose by the glucose oxidase, characterised in that the glucose oxidase and the peroxidase are present in proportions which provide from 300 to 700 International Units (IUs) of glucose oxidase and at least 20 International Units of peroxidase and in that
15 the chromogen is present in an amount which provides from 12 to 20 grams of active chromogen per 500,000 International Units of glucose oxidase. Preferably, the glucose oxidase is present in from 400 to 550 IUs per 27.5 to 32.5 IUs of peroxidase and the chromogen is o-tolidine which is present in an amount of from
20 12 to 15 gs per 500,000 IUs of the glucose oxidase.

It is preferred that the reagent mixture be put up in a gel matrix, notably a gelatin matrix, which provides from 200 to 400 gs of the matrix on a dry weight basis per 500,000 IUs of
25 the glucose oxidase.

It is particularly preferred that the reagent mixture/matrix be absorbed in a micro-porous membrane carrier.

30 The enzymatic reagents as used herein can be present in any suitable form, for example as the dry powdered active enzyme or as a precursor or addition product thereof which under the conditions of the test to be carried out produces an active enzyme in the reagent mixture. Thus, the enzymatic reagent can
35 be an active enzyme, for example glucose oxidase or peroxidase, or a stabilised form thereof, for example an acetate or other

salt or adduct thereof, which releases the active enzyme when the reagent mixture is wetted.

For convenience, the invention will be described hereinafter in terms of a mixture of glucose oxidase and peroxidase as conventionally used in the assessment of glucose in a blood sample.

Similarly, the term chromogenic material is used herein to denote any material which develops a property upon interaction with one or more of the products produced when the enzymatic reagent reacts with the blood sample to be assessed. Thus, the term includes materials which develop ultraviolet fluorescence or other detectable but not visible properties. However, it is preferred that the chromogenic material be one which develop a colour within the visible spectrum, for example as when di-anisidine or o-tolidine reacts with the hydrogen peroxide released when glucose in blood interacts with the glucose oxidase in the reagent mixture. The chromogenic material can be used in the form of the active material or a precursor or adduct thereof, notably an inorganic acid salt thereof such as the hydrochloride or sulphate, which releases the active ingredient during the test.

For convenience, the invention will be described hereinafter in terms of o-tolidine as the chromogenic material.

The enzymatic reagent and chromogenic material are operatively associated with one another so that they can interact under the conditions of the test procedure. Typically, the reagent and the chromogenic material will be put up in physical admixture with one another. However, it is within the scope of the present invention to put up the reagent and chromogenic material in a two part form which is admixed immediately prior to use; or in a form in which the reaction products of the interaction of the enzyme reagent with the glucose in the blood

sample diffuse into a zone containing the chromogenic material to develop the colour therewith separately from the enzyme interaction zone. Thus, for example, the enzyme reagent can be concentrated at one end or one side of a reagent pad and the
5 chromogenic material at the other end or side.

For convenience, the invention will be described hereinafter in terms of a pad of the reagent mixture containing the enzymatic reagent and the chromogenic material substantially uniformly
10 distributed throughout the pad.

The reagent mixture may contain other materials as is customary, for example phosphate buffering agents, preservatives, anti-coagulants or surface active agents. Such
15 other materials are typically inert to the material to be tested, the other constituents of the reagent mixture and the products of the reactions and interactions which occur during the test procedure. Such other constituents can be present in the amounts normally used in such reagent mixtures.

20 As stated above, we have found that if the enzymatic reagent and chromogenic material are present in the reagent mixture composition within specified proportions, the colour which the interactions between the material being assessed and the
25 various components of the mixture is surprisingly stable and enables the colour to be observed over a wider period of time than hitherto. Thus, the enzymatic reagents will typically be present in proportions of from 400 to 600, notably 450 to 550, International Units (IUs) of glucose oxidase and at least 20
30 IUs, typically about 27.5 to 35 IUs of peroxidase in the mixture. The chromogenic material will typically be present in an amount of from 12 to 17, notably from 13 to 16, grams per 500,000 IUs of the glucose oxidase. The optimum proportions within these ranges can be determined for any given case and a
35 given carrier by simple trial and error tests.

As stated above, the reagent mixture is preferably put up in a matrix carrier medium so that the material to be assessed can penetrate to the enzyme reagent and the chromogenic material. The matrix can be provided by a natural gum, jelly or gel, for example a gelatin, agar agar, aspic or silica gel; or can be provided by a synthetic polymer gel, for example a cellulosic gel or a polyvinyllic resin gel. For convenience, the invention will be described hereinafter in terms of the use of a gelatin gel as the carrier matrix.

10 The gel matrix can carry the enzymatic reagent and chromogenic material substantially uniformly distributed throughout it. This is conveniently achieved by mixing the enzyme reagents into a premix of the gelling agent and the chromogenic material; and allowing the mixture to set in the desired form. 15 Alternatively, the enzyme reagent and the chromogenic material can be admixed with a thixotropic gel carrier which is worked, for example by being stirred, to maintain it in the fluid state during mixing, but which is then allowed to set for storage and 20 transport prior to use.

Alternatively, the matrix may contain the enzymatic reagent and chromogenic material non-uniformly distributed therein, as when a gel layer is formed which has a high concentration of the gel 25 matrix in its upper layers to act a protective layer or coating for the reagent rich lower layers; or where the enzymatic reagent is located in a separate zone of the matrix from that containing the chromogenic material. In this case, the product from the interaction of the material being tested with one or 30 more of the enzymatic reagents diffuses from the enzyme zone into the zone containing the chromogenic material to develop a colour as a separate stage in that zone.

For convenience, the invention will be described hereinafter in terms of a reagent mixture which is uniformly distributed 35 throughout a gelatin matrix.

The matrix carrying the reagent mixture can be put up in a number of physical forms, for example as test strips or discs in which a pad of the matrix is applied to one face of the strip or disc and the colour resulting from the interaction of the material under assessment and the reagents and materials in the matrix is observed visually against the white background of the support strip or disc or against a separate reference background. Alternatively, the matrix can be put up in a series of zones through which the reagent mixture is distributed so that the interaction of the material to be assessed with the enzyme occurs in one zone and the product of that interaction diffuses to a second zone in which the colour reaction takes place. In a further alternative, the reagent mixture matrix can be absorbed or impregnated into the pores of a porous carrier to one face of which the material to be assessed is applied and the colour developing within the matrix is viewed from the opposite surface of the carrier.

For convenience, the invention will be described in terms of the use of a pad or disc of a micro-porous membrane which is impregnated with the reagent matrix.

In a conventional blood test reagent mixture, the gel matrix is a high molecular weight gelatin which is present in about 4% by dry weight. However, where the reagent mixture is to be absorbed into a micro-porous membrane, we prefer to use a low molecular weight gelatin, typically with a molecular weight in the range 20,000 to 50,000. Where such a gelatin is present in the amounts used hitherto, we have found that this results in a gel matrix which cannot be held satisfactorily within the pores of the membrane. On the other hand, we have found that if the gel content of the reagent mixture composition exceeds about 20% by dry weight, the gel inhibits the diffusion of reaction products through the membrane and hence development of a colour reflecting the true extent of the interactions which have occurred. We therefore prefer to provide the gel matrix

as a low molecular weight gelatin in an amount of from 250 to 325 gs by dry weight per 500,000 IUs of the glucose oxidase present in the reagent mixtrue.

- 5 The invention will now be illustrated by the following Example in which all parts and percentages are given by weight unless stated otherwise.

10 A first solution was made by stirring together at room temperature 300 mls of de-ionised water, 200 mls of 0.5 Molar sodium phosphate buffer to give a pH of 7, 100 mls of a 20% w/v solution of the surfactant Gantrez and 300 gs of dry powdered gelatin having a molecular weight in the range 25,000 to 40,000.

15 A second solution was prepared by stirring together at 60° C for one hour 300 mls of de-ionised water, 300 mls of methoxyethanol and 15 gs of o-tolidine hydrochloride or dianisidine hydrochloride.

20 The second solution was mixed dropwise with stirring into the first solution and the mixture stood for 1 hour at 60° C.

25 A third solution was made up by mixing 500,000 IUs of glucose oxidase and 30,000 IUs of peroxidase in a 0.1 Molar solution of the sodium phosphate buffer. This solution was mixed with stirring into the other mixed solutions and filtered through a 0.1 micrometre aperture filter.

30 The resultant solution was impregnated into a polysulfone resin sheet (0.2 to 0.4 mm thick and having an average pore diameter of 0.2 micrometres and an air permeability of 3 litres per minute per square centimetre at an applied pressure of 10 psig) to provide 5 IUs of glucose oxidase, 3 IUs of peroxidase, 0.2
35 milligrams of o-tolidine and 4 milligrams of gelatin per square centimetre of the membrane.

By way of comparison, the same membrane was impregnated with a conventional reagent mixture to provide the conventional level of enzyme and chromogen per square centimetre.

- 5 Blood samples were applied to the faces of a number of 6 mms diameter discs cut from each of the membranes. With the reagent compositions of the invention, a blue colour developed after only 10 seconds. The hue and intensity of the colour became stable after about 30 to 40 seconds and remained stable
10 for a further 30 to 40 seconds, thus allowing considerable latitude for the time to observe the colour. By way of comparison, the conventional formulations gave a colour which deepened in hue and intensity over 10 to 30 seconds after applying the blood sample, but which degenerated after a
15 further 15 seconds, giving little or no latitude in the time for observing the true colour.

- From another aspect, the present invention provides a method for making a test reagent mixture of the invention, wherein the
20 components of the mixture are admixed with one another to provide a substantially uniform mixture of the components.

- The invention further provides a method for making a test reagent mixture carried on a micro-porous carrier medium,
25 wherein a fluid reagent mixture of the invention is applied to the carrier medium. Preferably, the mixture is applied by impregnating the medium, for example by padding a sheet of the carrier through a bath of the reagent mixture, and allowing the mixture to gel within the pores of the carrier. Preferably,
30 the gelled mixture blinds the bores of the pores of the carrier so that rupture of blood or other cells due to capillary action by the pores is reduced. Discs or other shapes can be cut from the impregnated carrier for mounting on tests sticks having apertures therein or as the end walls of sample receivers so
35 that the colour which develops in the carrier can be observed from the opposite side to that to which the blood is applied.

CLAIMS:

1. A blood test reagent mixture composition comprising the enzymes glucose oxidase and peroxidase and a chromogen which
5 interacts with the hydrogen peroxide from the oxidation of the blood glucose by the glucose oxidase, characterised in that the glucose oxidase and the peroxidase are present in proportions which provide from 300 to 700 International Units (IUs) of
10 glucose oxidase and at least 20 International Units of peroxidase and in that the chromogen is present in an amount which provides from 12 to 20 grams of active chromogen per 500,000 International Units of glucose oxidase.
2. A test reagent mixture as claimed in claim 1,
15 characterised in that the glucose oxidase is present in from 400 to 550 IUs per 27.5 to 32.5 IUs of peroxidase and the chromogen is o-tolidine which is present in an amount of from 12 to 17 gs per 500,000 IUs of the glucose oxidase.
- 20 3. A test reagent mixture as claimed in either of claims 1 or 2, characterised in that it is put up in a gel matrix.
4. A test reagent mixture as claimed in claim 3,
characterised in that the gel matrix is a gelatin matrix, which
25 provides from 200 to 400 gs of gelatin on a dry weight basis per 500,000 IUs of the glucose oxidase.
5. A test reagent mixture as claimed in any one of the preceding claims, characterised in that the reagent mixture is
30 carried by a micro-porous membrane.
6. A test reagent mixture as claimed in either of claims 4 or 5, characterised in that the gelatin has a molecular weight in the range 20,000 to 50,000 and is present in an amount of from
35 250 to 325 gs by dry weight per 500,000 IUs of the glucose oxidase.

7. A test reagent mixture according to claim 1, substantially as hereinbefore described in the Example.

8. A method for making a test reagent mixture as claimed in claim 1, characterised in that the components of the mixture are admixed with one another to provide a substantially uniform mixture of the components.

9. A method for making a test reagent mixture as claimed in claim 5, characterised in that a fluid reagent mixture as claimed in any one of claims 1 to 4 or claim 6 is impregnated into the pores of a micro-porous carrier membrane.

10. A method for testing blood samples, characterised in that it comprises applying blood to a test reagent mixture as claimed in claim 1 and observing the colour which develops.

11. A method as claimed in claim 10, characterised in that the reagent mixture is carried by a micro-porous carrier membrane and the blood is applied to one face of the membrane and the colour is observed from the opposite face of the membrane.

25

30

35

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 92/01116

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12Q1/54; C12Q1/26; C12Q1/28		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12Q	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US,A,4 340 669 (R. BAUER) 20 July 1982 see the whole document	1-11
A	EP,A,0 016 947 (BOEHRINGER MANNHEIM GMBH.) 15 October 1980 see the whole document	1-11
A	FR,A,2 295 425 (BOEHRINGER MANNHEIM GMBH.) 16 July 1976 see the whole document	1-11
A	GB,A,893 318 (MILES LABORATORIES, INC.) 4 April 1962 see the whole document	1-11
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
02 OCTOBER 1992	17 5. 10. 92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	GRIFFITH G. <i>Gerard Griffith</i>	

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. GB 9201116
SA 60990**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 02/10/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4340669	20-07-82	AT-T- 9821	15-10-84
		AU-B- 524238	09-09-82
		CA-A- 1157749	29-11-83
		EP-A, B 0058334	25-08-82
		JP-C- 1311966	11-04-86
		JP-A- 57146599	10-09-82
		JP-B- 60035118	13-08-85
		US-A- 4391905	05-07-83
		US-A- 4391906	05-07-83
EP-A-0016947	15-10-80	DE-B- 2913553	09-10-80
		AT-T- 4126	15-07-83
		AU-B- 515851	07-05-81
		AU-A- 5677980	09-10-80
		CA-A- 1152871	30-08-83
		JP-C- 1111586	31-08-82
		JP-A- 55135599	22-10-80
		JP-B- 56046800	05-11-81
		US-A- 4517287	14-05-85
FR-A-2295425	16-07-76	DE-A- 2460903	24-06-76
		AT-B- 350189	10-05-79
		AU-B- 504794	01-11-79
		AU-A- 8755775	23-06-77
		BE-A- 836789	18-06-76
		CA-A- 1060906	21-08-79
		CH-A- 619048	29-08-80
		DE-C- 2462952	01-10-87
		GB-A- 1464360	09-02-77
		GB-A- 1464359	09-02-77
		JP-A- 54036237	16-03-79
		JP-C- 1263517	16-05-85
		JP-A- 51089491	05-08-76
		JP-B- 54003394	22-02-79
		NL-A- 7514628	23-06-76
		SE-B- 458687	24-04-89
		SE-A- 7514196	22-06-76
		US-A- 4385114	24-05-83
GB-A-893318		US-A- 3012976	

THIS PAGE BLANK (USPTO)